ECOLOGICAL SOIL SCREENING LEVELS FOR PLANTS EXPOSED TO TNT: SUPPORTING RANGE SUSTAINABILITY FOR TRAINING AND TESTING

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ABSTRACT

Ecological Soil Screening Levels (Eco-SSL) are those screening concentrations of chemicals in soil that present an acceptable risk to ecological receptors. We investigated the ecotoxicity of TNT to the plant species alfalfa (*Medicago sativa* L.), Japanese millet (*Echinochloa crusgalli* L. (Beauv.)), and perennial ryegrass (*Lolium perenne* L.) in five natural soils: Sassafras sandy loam (SSL), Teller sandy loam (TSL), Richfield clay loam (RCL), Kirkland clay loam (KCL), and Webster clay loam (WCL). According to USEPA Eco-SSL criteria, relative bioavailability scores for organic chemicals in these soils were rated "high" for SSL and TSL, "medium" for RCL and KCL, and "low" for WCL soil. We amended TNT into these soils and subjected them to wetting/drying cycles (8 weeks) in order to represent field conditions. Phytotoxicity studies were conducted with each soil separately in environmentally controlled growth chambers. Nonlinear regression models were used to determine 20% (EC_{20}) or 50% (EC_{50}) effect concentration values for seedling emergence, fresh mass, and dry mass. The geometric mean of EC_{20} values was used to determine draft Eco-SSL for each soil type. Results of these studies will undergo quality assurance before inclusion in the U.S. EPA national Eco-SSL database.

1. INTRODUCTION

The U.S. Army Strategy for the Environment (U.S. Army ASAIE, 2004) mandates that to sustain the future Army we must implement effective policies and practices that safeguard the environment and our quality of life. Sustainability connects our activities today to those of tomorrow with sound business and environmental practices. This strategy will use innovative technology and the principles of sustainability to enhance joint operation capability, meet current and future training and testing requirements, improve our ability to operate installations, reduce costs, and minimize impacts so that the Army can do more, and do it efficiently. In order to obtain these goals, knowledge about potential fate and effects resulting from testing and training with explosives such as 2,4,6-trinitrotoluene (TNT) in different field environments is essential to ensure Warfighter safety and environmental sustainability.

Objective Force Soldiers must receive highly realistic training across the spectrum of military operations. Realistic training leads to increased releases of energetic materials such as TNT into the environment at training and testing sites. Concentrations of TNT in soil have been reported to exceed 87,000 mg kg^{-1} (Simini et al., 1995). TNT contamination at these sites may therefore pose significant risk to DOD personnel and the surrounding environment. Examination of the toxic effects of TNT on plants in different soil types provides valuable information about the extent of environmental impact and potential food chain effects. Knowledge acquired from these studies will be used to quantify ecotoxicological benchmarks to develop Ecological Soil Screening Levels (Eco-SSL). Eco-SSL are those concentrations of chemicals in soil that present an acceptable risk to ecological receptors (USEPA, 2005). The studies described herein address existing data gaps, and establish ecotoxicological benchmarks that are necessary to derive TNT Eco-SSL for plants, relative to soil type.

2. METHODS

Five soils, Sassafras sandy loam (SSL), Teller sandy loam (TSL), Richfield clay loam (RCL), Kirkland clay loam (KCL), and Webster clay loam (WCL), that differ in relative bioavailability for organic chemicals, due to different physical and chemical properties (Table 1), were amended with TNT. We adapted standardized toxicity tests (ASTM, 1998) and conducted bioassays with three plant species after subjecting the TNT in

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Table 1. Properties of "A" horizon of amended field soils. CEC = cation exchange capacity.

Soil parameter	Teller	Sassafras	Richfield	Kirkland	Webster
Sand (%)	65	69	29	37	33
Silt (%)	22	13	42	34	39
Clay (%)	13	17	29	29	28
Texture	sandy loam	sandy loam	clay loam	clay loam	clay loam
CEC (cmol/kg)	4.3	5.5	28	10	21
Organic Matter (%)	1.4	1.3	3.3	2.6	5.3
pH	4.4	5.2	7.4	6.4	5.9

the amended soils to 8 weeks of wetting/drying cycles, thereby weathering and aging the TNT within the soils. The weathering and aging process was conducted to simulate field conditions.

Results from range-finding tests (preliminary data; not shown) were used to determine the range of concentrations for definitive plant toxicity testing for each of the five soil types used in this study. TNT was first dissolved in acetone, then added to each soil, dried in darkness overnight to evaporate the acetone, and thoroughly mixed into the soil using a three-dimensional soil mixer to yield nominal concentrations of 1, 10, 20, 40, 80, 160, 320 and 640 mg kg^{-1}. The TNT amended into these soils was then subjected to a weathering and aging process in a greenhouse for 8 weeks (hydrated once a week to 60% water-holding capacity, and allowed to dry). TNT concentrations in soil were measured by HPLC (using a modification of EPA Method 8330) after the weathering and aging process and before toxicity tests. TNT phytotoxicity studies were then conducted with each soil series separately in environmentally-controlled growth chambers at 22$\pm$2ºC, 16h light / 8h dark cycles, with >60% relative humidity. Negative, solvent, and positive (boric acid) control treatments were included in each test. Percentage of seedlings emerging (seedling emergence) was measured after 5 to 7 d, and fresh and dry masses of shoots were measured after an additional two-week growth period. We used nonlinear regression models to determine the EC_{20} and EC_{50} values (soil concentrations causing 20% or 50% reduction, respectively, compared to solvent controls) for seedling emergence, shoot fresh mass, and shoot dry mass. Results from negative controls were used to determine compliance with standardized protocols, and positive controls for compliance with laboratory testing norms. Draft Eco-SSL were derived by calculating the geometric mean of EC_{20} values for both the fresh and dry shoot mass endpoints of the three test species (USEPA, 2005).

3. RESULTS AND DISCUSSION

Results of these tests are shown in Table 2. Weathered and aged TNT reduced both shoot fresh and dry masses in alfalfa, Japanese millet, and perennial ryegrass in all soil types compared to control treatment (Table 2). Toxicity test results were fitted using regression analyses for [1] Linear, [2] Gompertz (logistic), [3] Exponential, and [4] Hormetic (logistic with growth stimulation at low concentrations) models (Figure 1).

[1] Linear Model

$$Y=[(-bp)/EC_p] \times C + b$$

[2] Gompertz Model

$$Y = a \times e([\log(1\text{-}p)] \times [C/ECp]^b)$$

[3] Exponential Model

$$Y = a \times e(([\log(1\text{-}p)] / ECp) \times C) + b$$

[4] Hormetic Model

$$Y = (t \times [1 + hC]) /\{1 + [(p + h\, ECp) / (1 - p)] \times [C/ECp]^b\}$$

Where :

Y = number for a measurement endpoint (e.g., number of juveniles)
a = control response
t = control response in the hormetic model
e = base of the natural logarithm
p = percent inhibition/100 (e.g., 0.50 for EC_{50})
C = exposure concentration in test soil
ECp = estimate of effect concentration for a specified percent effect
h = hormetic effect parameter
b = scale parameter

Weathered and aged TNT was toxic to all plant species in all soil types. Toxicity was less (greater EC_{20} and EC_{50} values) in plants exposed to TNT in Webster clay loam, compared to the other soil types (Table 2). Seedling emergence, and shoot fresh or dry masses, were not significantly (95% C.I.) different among TSL, SSL, KCL, and RCL soils (Table 2). The Linear model was the most common best-fit for seedling emergence and the Hormetic model was most commonly best-fit for shoot fresh and dry masses (Table 2; Figure 1). Shoot fresh

Table 2. Summary of provisional toxicological benchmark concentrations for TNT, weathered and aged in Teller sandy loam (TSL), Sassafras Sandy Loam Soil (SSL), Richfield clay loam (RCL), Kirkland clay loam (KCL), and Webster clay loam (WCL) determined for alfalfa, Japanese millet, and ryegrass.

	Seedling emergence			Shoot fresh mass			Shoot dry mass		
	MODEL	EC_{20} (mg kg^{-1})	EC_{50} (mg kg^{-1})	MODEL	EC_{20} (mg kg^{-1})	EC_{50} (mg kg^{-1})	MODEL	EC_{20} (mg kg^{-1})	EC_{50} (mg kg^{-1})
Alfalfa									
TSL	Linear	54 (44-64)	135 (109-161)	Hormetic	12 (1-22)	36 (10-62)	Hormetic	18 (3-33)	42 (3-81)
SSL	Gompertz	68 (40-96)	84 (77-91)	Exponential	7 (4-11)	22 (12-33)	Exponential	10 (4-16)	31 (13-49)
RCL	Linear	43 (26-61)	108 (64-152)	Hormetic	21 (8-33)	33 (20-46)	Hormetic	8 (3-13)	22 (1-43)
KCL	Linear	40 (28-52	99 (70-129)	Hormetic	9 (4-13)	20 (11-29)	Hormetic	13 (8-17)	26 (17-34
WCL	Linear	254 (143-365)	635 (358-911)	Hormetic	114 (80-149)	200 (129-270)	Hormetic	113 (79-148)	206 (135-278)
Japanese millet									
TSL	Linear	65 (55-76)	163 (137-190)	Hormetic	21 (7-34)	40 (14-65)	Gompertz	28 (6-50)	56 (32-79)
SSL	Gompertz	67 (35-99)	169 (131-206)	Hormetic	5 (4-6)	8 (6-9)	Hormetic	6 (5-7)	10 (8-12)
RCL	Linear	68 (52-85)	171 (129-212)	Hormetic	15 (0-32)	26 (17-36)	Hormetic	11 (0-23)	22 (14-31)
KCL	NS	NS	NS	Hormetic	12 (9-14)	16 (13-20)	Hormetic	12 (10-14)	20 (18-23)
WCL	Linear	266 (190-343)	666 (475-858)	Hormetic	105 (89-122)	157 (130-183)	Hormetic	99 (86-111)	147 (128-167)
Perennial ryegrass									
TSL	Gompertz	58 (41-76)	92 (80-105)	Exponential	8 (4-12)	24 (12-37)	Gompertz	5 (0-10)	23 (11-36)
SSL	Gompertz	27 (16-38)	56 (45-67)	Hormetic	7 (5-8)	11 (9-13)	Hormetic	7 (5-8)	11 (8-14)
RCL	Linear	40 (35-45)	100 (88-113)	Hormetic	7 (2-12)	11 (9-13)	Exponential	9 (5-13)	28 (17-40)
KCL	Linear	44 (37-51)	110 (93-129)	Hormetic	10 (9-12)	16 (14-17)	Hormetic	12 (8-14)	20 (17-22)
WCL	Gompertz	339 (238-440)	480 (443-518)	Gompertz	137 (64-212)	184 (161-206)	Hormetic	127 (90-164)	185 (144-225)

Table notes: Effects concentrations at 20% (EC_{20}) and 50% (EC_{50}) were estimated from non-linear regression analysis using either linear, $Y=[(-bp)/IC_p]$ x $C + b$, Gompertz (logistic), $Y = a \times e([\log(1\text{-}p)] \times [C/\mathrm{ECp}]b)$, exponential, $Y = a \times e(([\log(1\text{-}p)] / \mathrm{ECp}) \times C) + \mathrm{b}$, or hormetic (logistic with low-level stimulation), $Y = (t \times [1 + hC]) /\{1 + [(p + h\ \mathrm{ECp}) / (1 - p)] \times [C/\mathrm{ECp}]b\}$ models. Confidence intervals (95%) are presented in brackets. NS = not significant (p< 0.05).

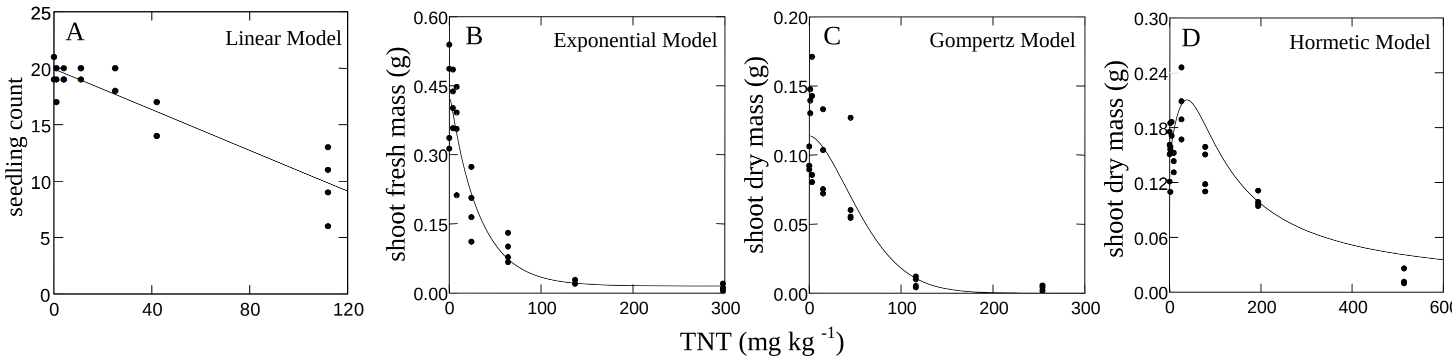

Figure 1. Representative regression curves for (A) Perennial ryegrass seedling emergence in KCL, (B) Alfalfa shoot fresh mass in SSL, (C) Japanese millet shoot dry mass in TSL, and (D) Alfalfa shoot dry mass in WCL.

and dry masses were more sensitive indicators of phytotoxicity than was seedling emergence (Table 2). Shoot dry mass of boric acid positive controls had EC_{50} values (95% CI) of 77 (48-105) mg kg^{-1} and 92 (38-146) mg kg^{-1}, respectively, in two separate tests performed within one year of the present study, showing that plant response was not significantly different over time, thus complying with ISO standard methods.

Draft Eco-SSL for plants (TNT mg kg^{-1} soil) calculated from these studies were 115, 13, 11, 11, and 6.8, for WCL, TSL, KCL, RCL, and SSL soils, respectively. The major property that distinguishes WCL from the others is organic matter content (OMC). The OMC of WCL is 5.3% compared to 3.3%, 2.6%, 1.4%, and 1.3% for RCL, KCL, TSL, and SSL, respectively (Table 1). WCL also had relatively high clay content (29%). Organic matter can bind organic chemicals in soil, thereby reducing their bioavailability (USEPA, 2005). Sorption is further enhanced in soils with relatively high clay content. Results of these studies will undergo quality assurance review before inclusion in the U.S. EPA national Eco-SSL database, as is required for all Eco-SSL benchmark data submitted to U.S. EPA.

CONCLUSIONS

Soil physical and chemical properties can alter the toxicity of TNT, as evidenced by the different ecotoxicological responses of plants exposed to the same TNT concentrations in different types of soil. Organic matter content appears to be the most prominent soil characteristic affecting TNT toxicity. Relatively high clay content within soils may also contribute to decreasing the toxicity of TNT. Such information can be used for environmental compliance, directly supporting the training of Objective Force Soldiers across training facilities at various locations that differ significantly in soil type. Environmental compliance may be met, in part, by both considering and taking advantage of the information derived from this study regarding the effects of soil characteristics on TNT phytotoxicity. This information can be beneficial when designing and scheduling field training and testing. When this information is effectively applied, field operations may proceed on schedule with greater efficiency while reducing environmental costs and improving sustainability of ranges for both training and testing.

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